Toxicity Studies on Natural Phytoplankton Assemblages by means of Fractionation Bioassays

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OF FISHERIES AND AQUATIC SCIENCES
No. 1152

DECEMBER, 1982

TOXICITY STUDIES ON NATURAL PHYTOPLANKTON ASSEMBLAGES BY MEANS OF FRACTIONATION BIOASSAYS

BY

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ABSTRACT

Munawar, M.. 1982. Toxicity studies on natural phytoplankton assemblages by means of fractionation bioassays. Can. Tech. Rep. Fish. Aquat. Sci., 1152 pages i - vi, 1 - 17.

The toxicity experiments were carried out in the Great Lakes during spring, summer and fall of 1979, 1980 and 1981. Algal fractionation approach was applied in the toxicity studies for the first time using natural phytoplankton assemblage. By means of Carbon-14 uptake fractionation, differential response of various size fractions, such as <10, 10 to 20, 20 to 64 and >64 µm, to various concentrations of a metal mixture was assessed. These results suggest that the nannoplantkon and particularly the smaller size fraction, <10 µm, was much more susceptible to inhibition by the mixture of heavy metals. Since it is also this group of algae that is preferred by herbivorous zooplankton, these results have strong implications for contaminant pathway management.

RESUME

Munawar, M. 1982. Toxicity studies on natural phytoplankton assemblages by means of fractionation bioassays. Can. Tech. Rep. Fish. Aquat. Sci., 1152. i-vi, 1-17 pp.

Les expériences toxicologiques ont été effectivées dans les Grands Lacs au printemps, en été en automne des années 1979, 1980 et 1981. Pour la première fois on a employé le methode des incubations avec ^{14}C suivi de la fractionation de la communauté phytoplanctonique pour l'estimation de l'inhibition de la photosynthèse par un melange des metaux lourds. Les resultats dans les différentes fractions (<10 µm, 10-20 µm, 20-64 µm et >64 µm) suggerent que le nanoplancton, et la classe la plus petite (<10 µm) en particulier, est manifestement le plus susceptible à l'inhibition par les metaux lourds. Etant donné que cette classe de taille est la nourriture prétérée du zooplancton herbivore, les implications pour l'aménagement du transfert des metaux son évidentes.

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INTRODUCTION

The limnological and phycological research in the St. Lawrence Great Lakes is at the crossroads of solution and problems. The eutrophication problem appears to be solved by means of a successful phosphorus control management strategy. However, increasing abundance of contaminants, with their associated toxicity to biota, confront efforts to preserve the Great Lakes.

The International Great Lakes Water Quality Agreements between the U.S.A. and Canada has therefore focussed on the rates and pathways of contaminants in the aquatic food chain (International Joint Commission, 1976). Similarly an ecosystem approach has been recommended by the Great Lakes Science Advisory Board (1978), for the protection of the aquatic biota. However, it is indeed surprising that most of the earlier toxicological work in the Great Lakes concentrated mainly on fish tissue burdens, with the exception of Wong *et al.* (1978) who evaluated the toxicity of a metal mixture on axenic cultures of algae. It seems that natural assemblages of organisms belonging to lower trophic levels, such as phytoplankton and zooplankton, have not been used in toxicological research in the Great Lakes. The potential use of lower trophic organisms, such as phytoplankton, is particularly important since these organisms play a key role as primary producers in the aquatic food chain and are grazed upon by the zooplankton. Since the impact of contaminants at this lower trophic level might be detrimental to the entire food chain, the bioassessment of these contaminants by means of AFB's could serve as an early and effective warning tool for contaminant management before they culminate as fish burdens.

The present report deals with an approach never used before in toxicology, namely, Algal Fractionation Bioassays (AFB's), which utilize various size fractions of natural phytoplankton communities as test organisms in toxicity testing. An extensive experimental programme was conducted in the Great Lakes, during the period 1979 - 1981, to evaluate the differential response of various size assemblages of phytoplankton to a mixture of metals. A few examples from the summer of 1980 are presented here to provide a comparative picture of the response of size assemblages of phytoplankton to contaminant stress.

METHODS

The toxicity experiments were performed in an incubator aboard the research ship CSS Bayfield. Integrated water samples were collected from 0 to 20 m (Schroeder, 1969) and divided into aliquots for taxonomic identification, chlorophyll-a determination, and toxicity experiments. The taxonomy sample was preserved in Lugol's solution and later analyzed by the settling-inverted microscope method (Munawar et al., 1974). After microscopic counts and measurements, the phytoplantkon biomass and species were partitioned into size classes by computer analysis (Table 2).

For each toxicity experiment subsamples were poured into four one-litre borosilicate glass bottles. One bottle was an untreated control and the other three were treated with different dosages (0.5X, 1.0X, and 2.0X Water Quality Objectives {W.Q.O.} levels) of a mixture of ten heavy metals. The 1.0X dosage represents the

recommended Water Quality Objectives level (the highest concentrations causing no significant harmful effect to the biota based on individual metals) for the Great Lakes (International Joint Commission, 1976). The details of the Water Quality Objectives for metal concentrations for the protection of the aquatic organisms and the scientific basis for the use of metal mixtures, instead of individual metals, has been discussed thoroughly by Wong et al. (1978).

Each bottle was then inoculated with 20 µCi (1 µCi equals 37 GBq) of $NaH\ ^{14}CO_3$. Since our primary objective was to study the impact of contaminants on the photosynthetic uptake of Carbon-14, and since previous experiments had shown dark uptake to be insignificant, dark bottles were not included. The bottles were incubated under a constant light intensity (30,000 lux) at the ambient lake temperature for four hours. Four replicate aliquots were then filtered from each bottle through a 0.45 µm Millipore membrane filter to estimate total community Carbon-14 uptake. Three other sets of four 50 ml aliquots were passed, respectively, through 64, 20 and 10 µm Nitex screens before filtration through the 0.45 µm filters. The filters were then acidified with 10 ml of 0.1N HCl and dissolved in Phase Combining System (PCS) for liquid scintillation counting (Lind and Campbell, 1969).

For the chlorophyll determinations, the same fractionation procedure was followed,, using Millipore membranes (Munawar et al., 1982), and the filters were treated with saturated $MgCO_3$ and frozen at -20° C for subsequent analysis by the spectrophotometric method of Strickland and Parsons (1968). The Carbon-14 uptake, biomass, and chlorophyll fractionation results have thus been

grouped and discussed in two categories: nannoplankton, <64 µm, and net plankton, >64 µm. The nannoplankton size fraction has been further categorized into 20 to 64 µm, 10 to 20 µm, and <10 µm size fractions (Munawar et al., 1978).

RESULTS & DISCUSSION

The abundance of nannoplankton in the phytoplankton assemblages of the Great Lakes has been emphasized several times (Munawar and Munawar, 1975; 1978; 1981). Their overwhelming contribution to primary production is well known (Munawar et al., 1978) and the important role they play in the transfer of contaminants via grazing has also been identified (Ross and Munawar, 1981). As a continuation of our ongoing research dealing with nannoplankton dynamics, fractionation of chlorophyll-a concentration was carried out on the samples which were used for the Carbon-14 Algal Fractionation Bioassays (AFB's). Table 1 gives the chlorophyll-a concentration and its percent composition by size. The data shows that a major portion of chlorophyll-a was contained in the nannoplankton, <64 µm, and µ-algae - ultraplankton, <10 µm, size fractions. Thus, nannoplankton in general and µ-algae - ultraplankton in particular are physiologically important since the photosynthetic pigments play a key role in reproduction as well as decomposition.

The results of the AFB's are discussed in two steps:

a) Nannoplankton - Netplankton fractions (Figures 1 to 4)

b) Nannoplankton fractions (Figures 5 to 7)

Figures 1 to 4 exhibit the results of the fractionation bioassays at various concentrations of the metal mixture, for the size fraction retained by a 64 µm Nitex screen (netplankton) and for the fraction passing through the net (nannoplankton). The netplankton uptake was relatively very low, while nannoplankton assimilated much more Carbon-14 in the control bottles, and was clearly affected by even the lowest metal mixture concentration in all of our experiments.

Another more detailed fractionation series was designed to look closely at the response of various components of the nannoplankton, namely; the 20 to 64 µm fraction, the 10 to 20 µm fraction, and the <10 µm (µ-algae and ultraplankton) size fraction. One example from each environment is given to provide a general comparison of the observed algal responses.

Figure 5 shows the response of the Lake Superior phytoplankton size assemblages at station 13 (off Keweenaw Point, see Fig. 4). The <10 µm (µ-algae and ultraplankton) was the most affected fraction, showing significantly high inhibition of Carbon-14 uptake. At station 13, 70 to 82 percent inhibition was shown by the <10 µm fraction which contributed 56 percent to the total phytoplankton biomass, as determined by microscopic enumeration, and contained 61.18 percent chlorophyll-a (Table 2). This size fraction also harboured the greatest number of species (28) which were dominanted by phytoflagellates such as Chrysochromulina parva Lackey, Uroglenopsis sp. and Chrysamoeba sp. (Table 2).

Figures 5 to 7 show the differential response of various size fractions of natural phytoplankton assemblages in the North Channel, Georgian Bay, Lake Huron, Saginaw Bay, and Western Lake

Erie respectively. The greatest inhibition of Carbon-14 uptake was consistently observed for the <10 µm size fraction. Other size fractions also suffered photosynthetic inhibition but did not show such dramatic inhibition as exhibited by the <10 µm fraction. Occasionally slight increases in Carbon-14 uptake were observed at higher metal concentrations although no consistent trends were observed.

In summary, our results suggest that nannoplankton in general, and the µ-algae and ultraplankton { <10 µm } in particular, are far more photosynthetically active size fractions than the net plankton and were much more susceptible to inhibition by contaminants such as a metal mixture. The dramatic and consistent inhibition of the <10 µm size fraction by the lowest concentration of the metal mixture employed is interesting and noteworthy since it is 50 percent lower than the concentration recommended in the Water Quality Objectives for the protection of Great Lakes biota.

Several implications could be deduced from our Algal Fractionation Bioassays (AFB's). First of all the recommended safe levels of heavy metals obviously are also not safe for natural phytoplankton assemblages when used synergistically, as concluded by Wong et al. (1978) for axenic cultures. Actually our results suggest that due to its sensitivity the <10 µm fraction could easily be used as a reliable testing tool, instead of cultures or total phytoplankton assemblages, in detecting the presence of toxic substances and as a screening technique for the bioavailability and toxicity of contaminants. Therefore Algal Fractionation Bioassays appear to have a tremendous potential as a routine technique for the bioassessment of the impact of contaminants since it is

sensitive, rapid, and inexpensive.

ACKNOWLEDGEMENTS

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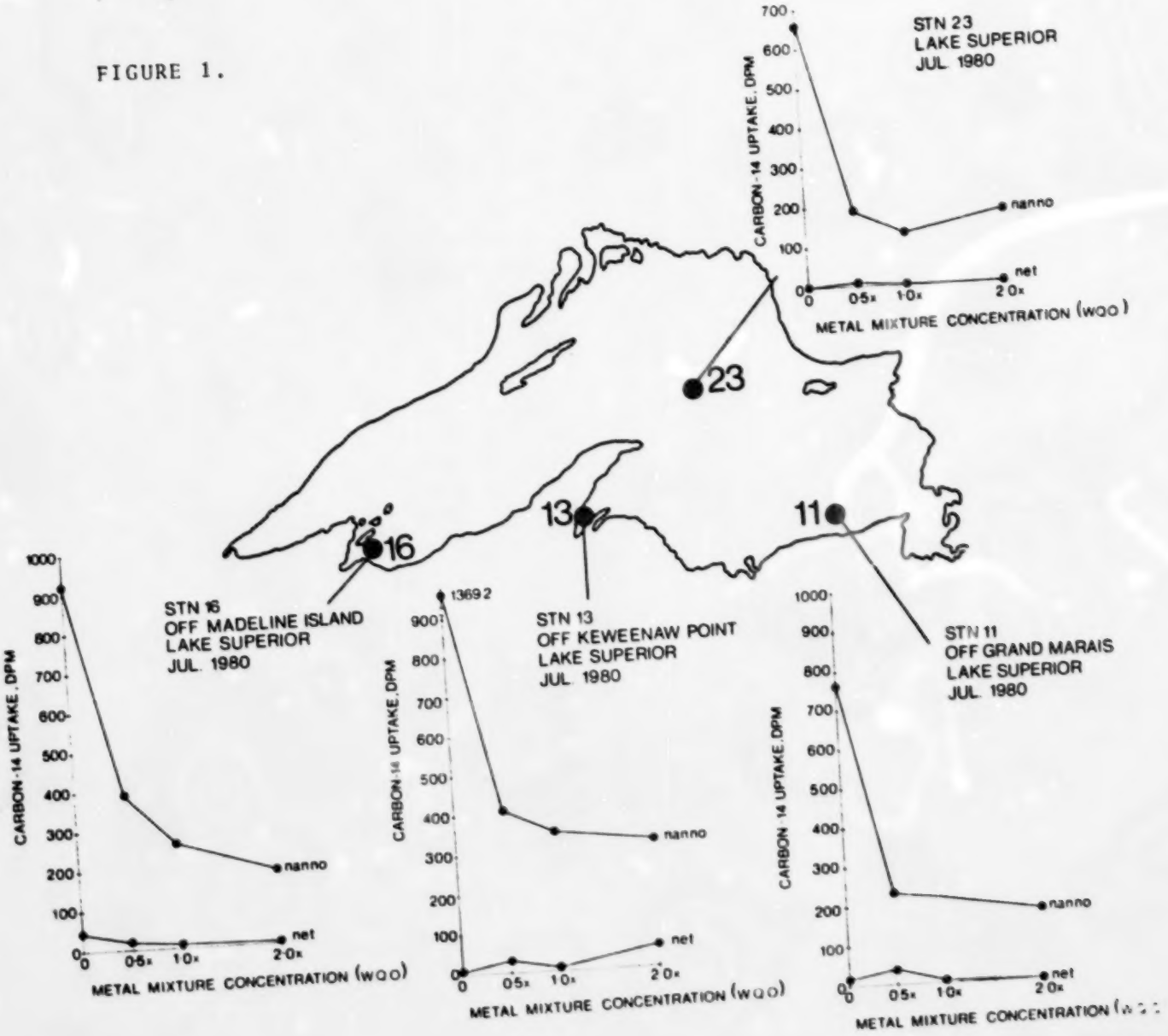
FIGURE 1.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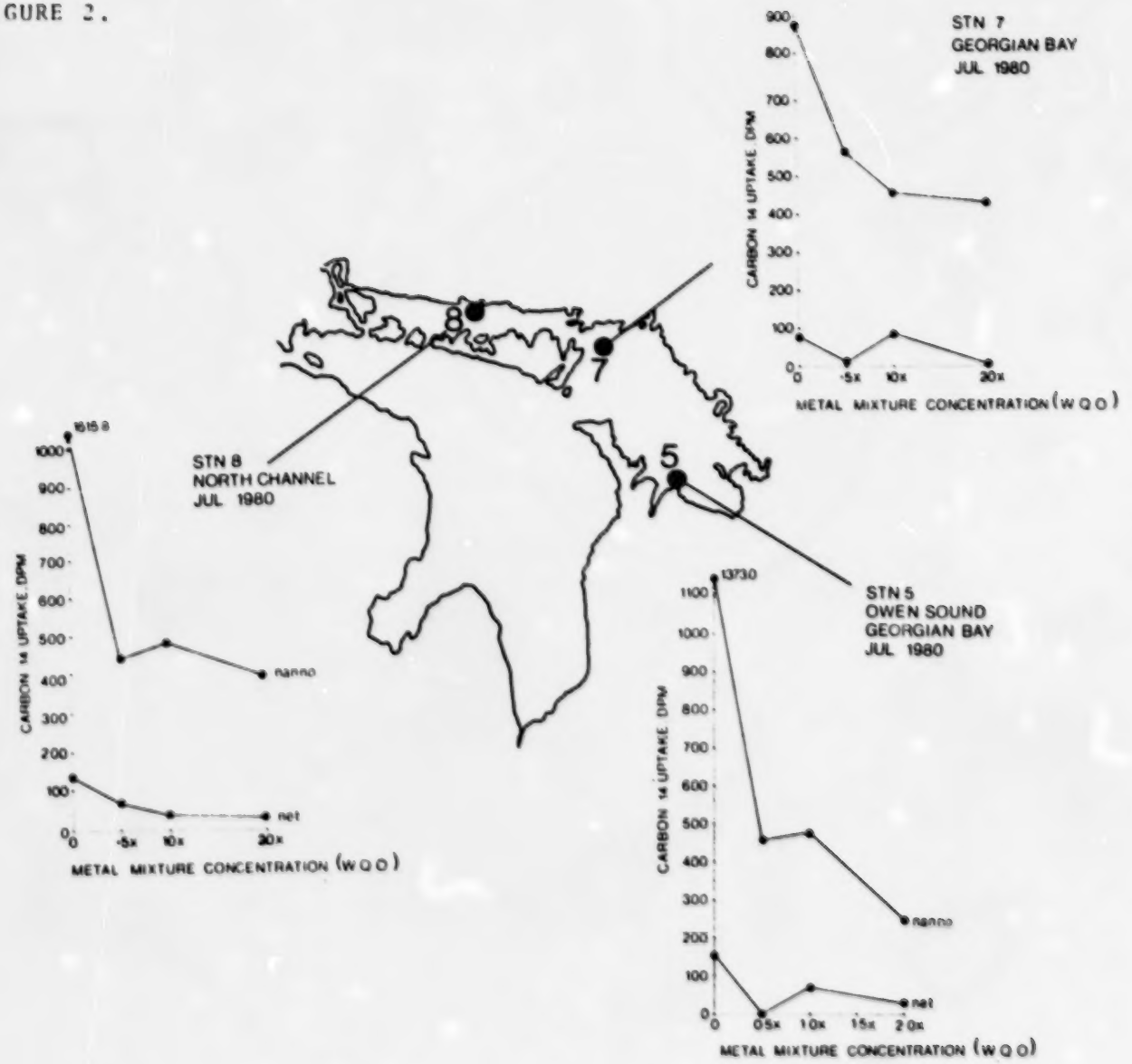
LAKE SUPERIOR
JUL. 1980
CARBON-14 UPTAKE.DPM
nanno
net
0
0.5x
1.0x
2.0x
METAL MIXTURE CONCENTRATION (WQO)
23
13
11
16
STN 16
OFF MADELINE ISLAND
LAKE SUPERIOR
JUL. 1980
STN 13
OFF KEWEENAW POINT
LAKE SUPERIOR
JUL. 1980
STN 11
OFF GRAND MARAIS
LAKE SUPERIOR
JUL. 1980

FIGURE 2.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GEORGIAN BAY
JUL 1980
CARBON 14 UPTAKE DPM
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NORTH CHANNEL
JUL 1980
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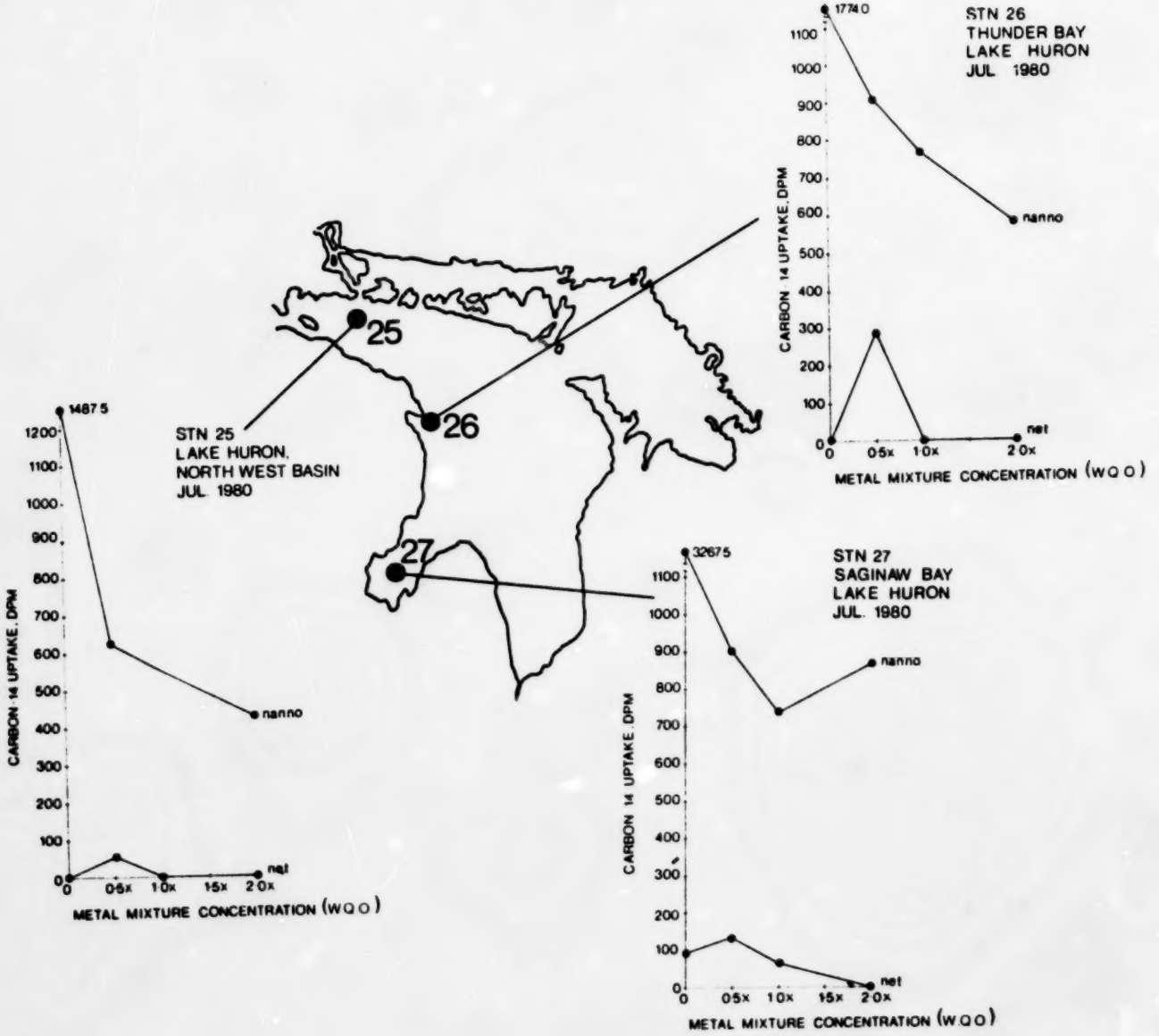
net
STN 5
OWEN SOUND
GEORGIAN BAY
JUL 1980
1373.0
nano
net

FIGURE 3.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LAKE HURON
JUL. 1980
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nanno
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CARBON 14 UPTAKE, DPM
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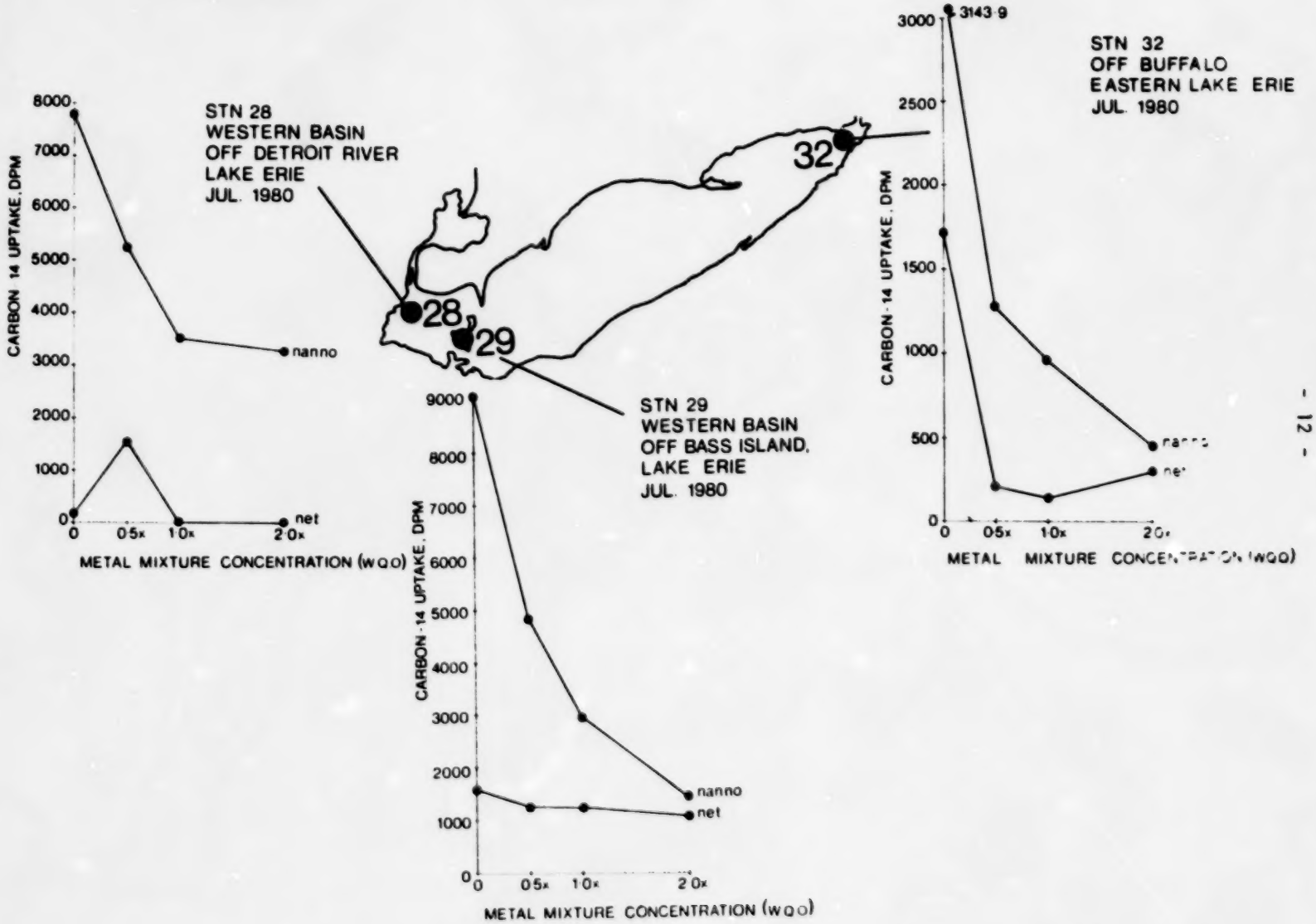
10x
20x
METAL MIXTURE CONCENTRATION (WQO)
25
26
27
STN 25
LAKE HURON,
NORTH WEST BASIN
JUL. 1980
14875
STN 27
SAGINAW BAY
LAKE HURON
JUL. 1980
32675
15x

FIGURE 4.


STN 28
WESTERN BASIN
OFF DETROIT RIVER
LAKE ERIE
JUL. 1980
CARBON-14 UPTAKE, DPM
8000
7000
6000
5000
4000
3000
2000
1000
0
nanno
net
0
05x
10x
20x
METAL MIXTURE CONCENTRATION (WQO)
28
29
32
STN 29
WESTERN BASIN
OFF BASS ISLAND,
LAKE ERIE
JUL. 1980
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
nanno
net
METAL MIXTURE CONCENTRATION (WQO)
STN 32
OFF BUFFALO
EASTERN LAKE ERIE
JUL. 1980
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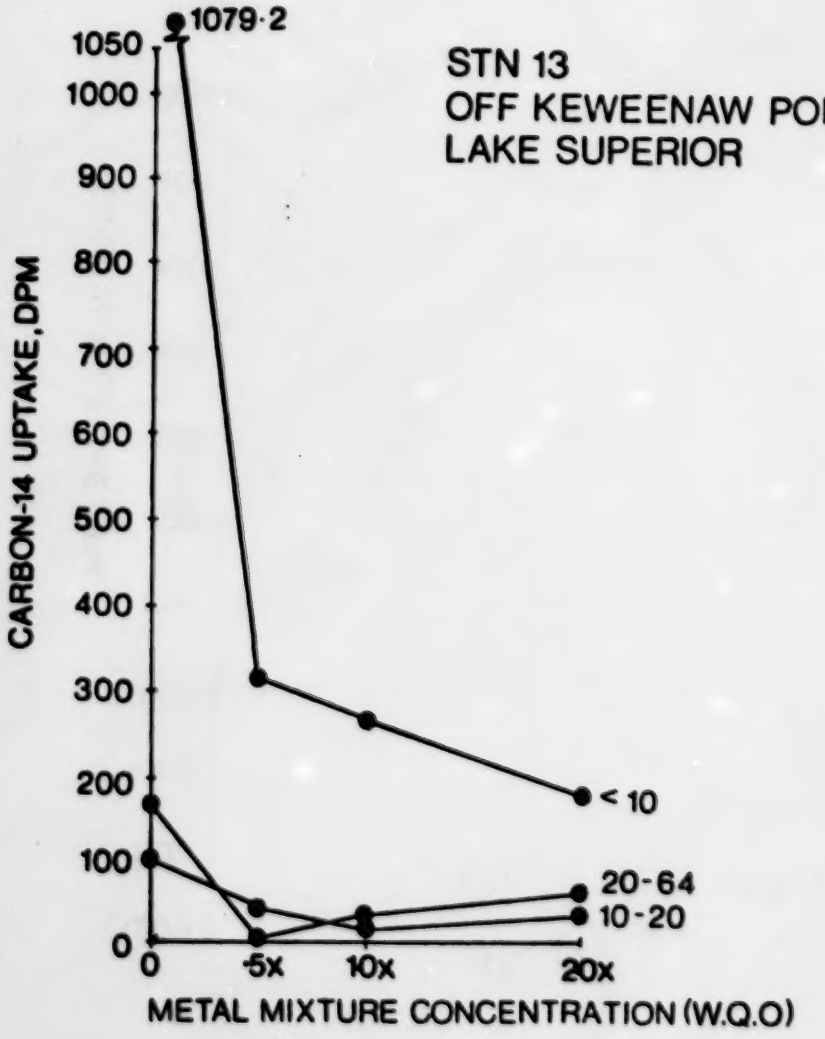
METAL MIXTURE CONCENTRATION (WQO)

FIGURE 5.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OFF KEWEENAW POINT
LAKE SUPERIOR
1079·2
CARBON-14 UPTAKE, DPM
1050
1000
900
800
700
600
500
400
300
200
100
0
< 10
20-64
10-20
0
·5x
10x
20x
METAL MIXTURE CONCENTRATION (W.Q.O)

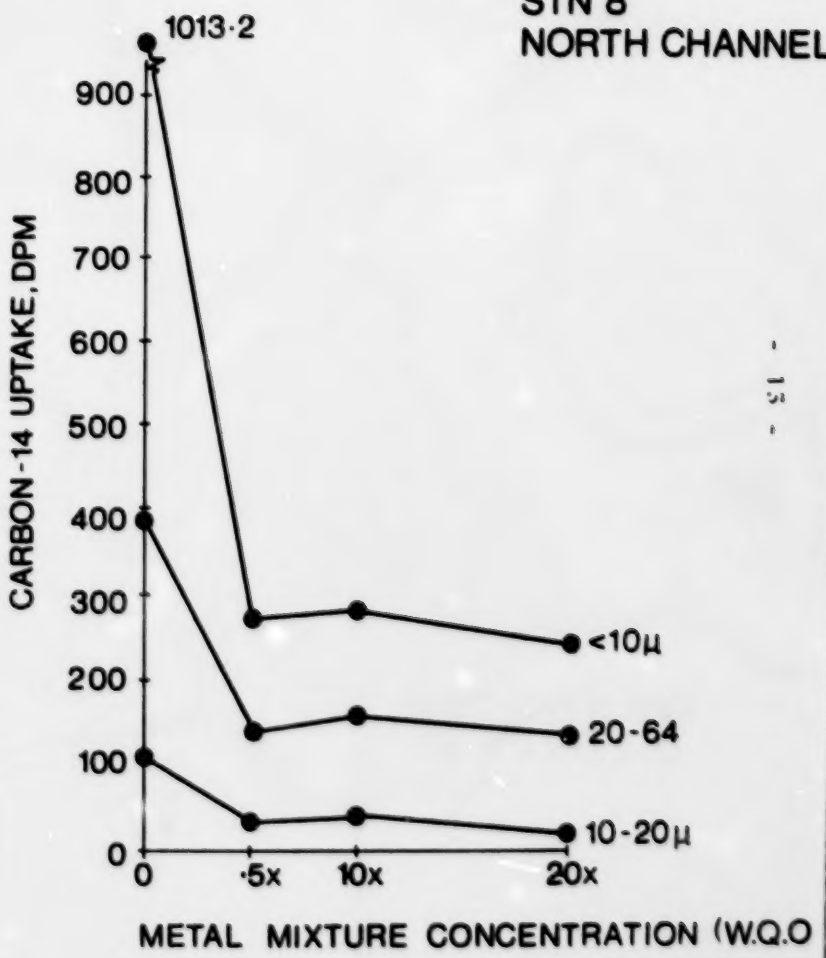
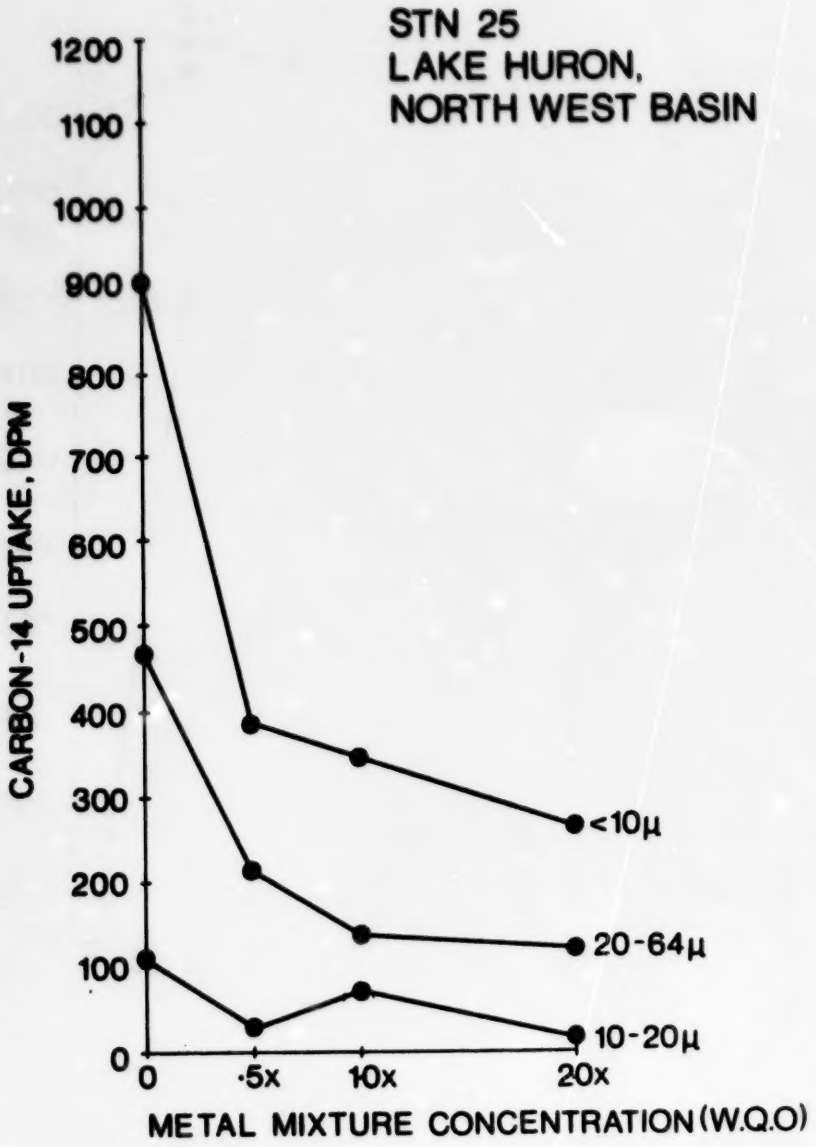

STN 8
NORTH CHANNEL
1013·2
CARBON-14 UPTAKE, DPM
900
800
700
600
500
400
300
200
100
0
<10µ
20-64
10-20µ
0
·5x
10x
20x
METAL MIXTURE CONCENTRATION (W.Q.O

FIGURE 6.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NORTH WEST BASIN
CARBON-14 UPTAKE, DPM
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
<10μ
20-64μ
10-20μ
0
·5x
10x
20x
METAL MIXTURE CONCENTRATION (W.Q.O)

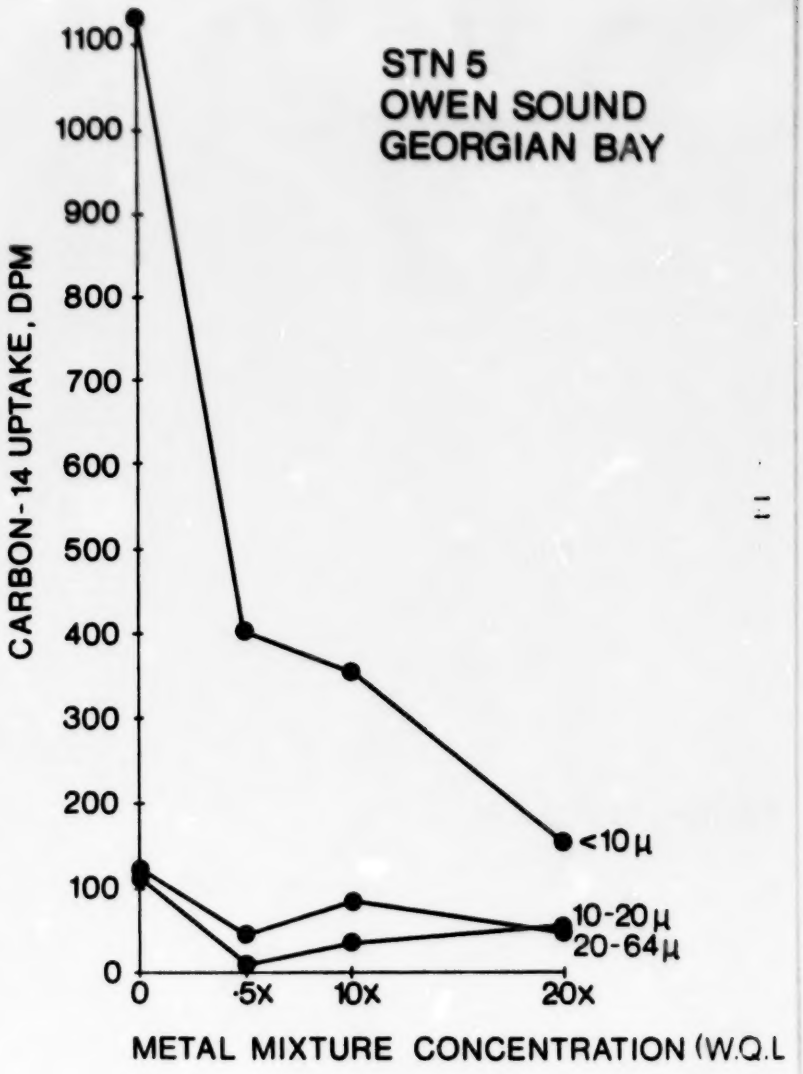
STN 5
OWEN SOUND
GEORGIAN BAY
CARBON-14 UPTAKE, DPM
1100
1000
900
800
700
600
500
400
300
200
100
0
<10μ
10-20μ
20-64μ
0
·5x
10x
20x
METAL MIXTURE CONCENTRATION (W.Q.L

FIGURE 7.

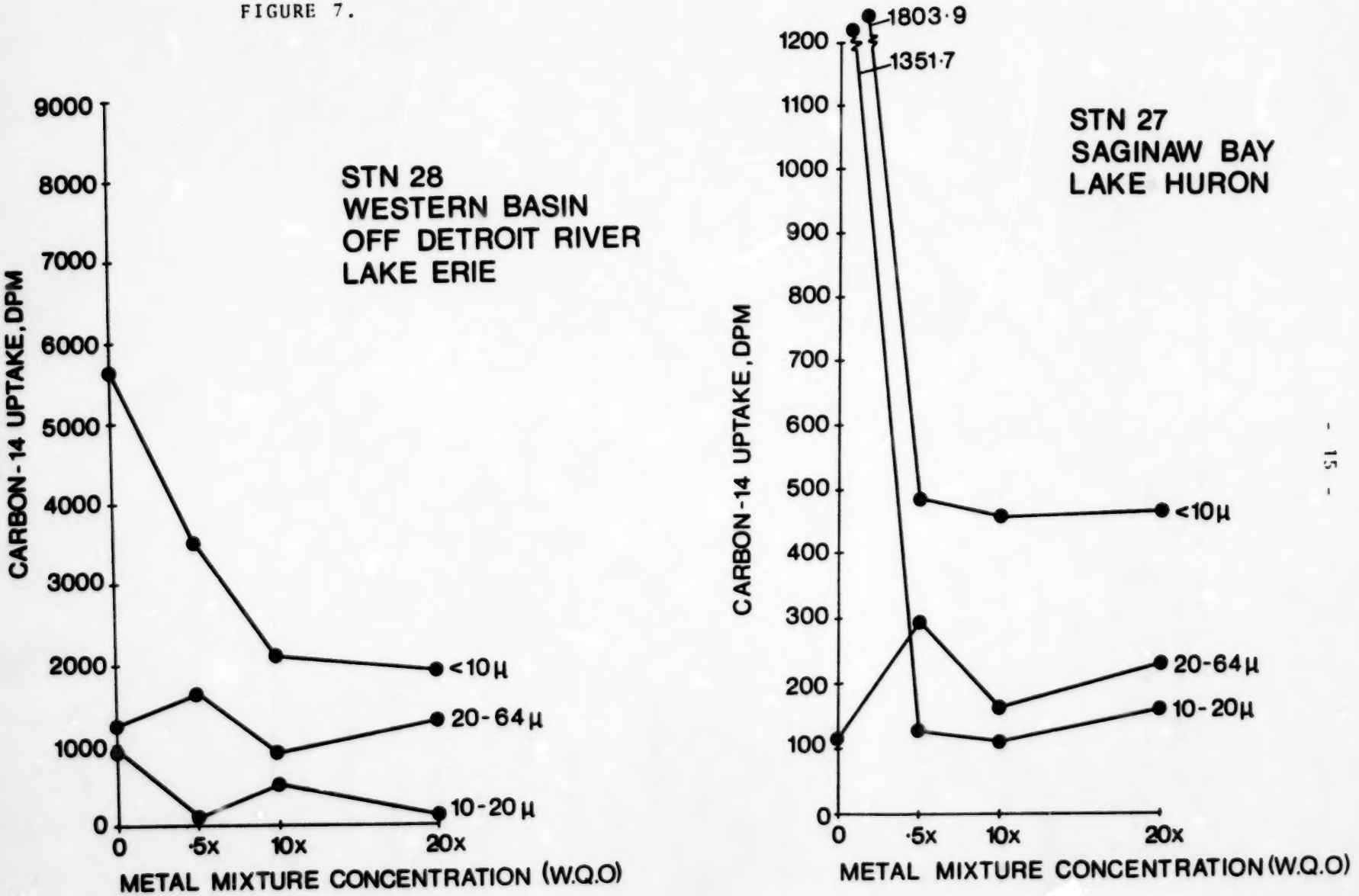
STN 28
WESTERN BASIN
OFF DETROIT RIVER
LAKE ERIE
CARBON-14 UPTAKE, DPM
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
<10μ
20-64μ
10-20μ
0
·5x
10x
20x
METAL MIXTURE CONCENTRATION (W.Q.O)
STN 27
SAGINAW BAY
LAKE HURON
1803·9
1351·7
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
<10μ
20-64μ
10-20μ
0
·5x
10x
20x
METAL MIXTURE CONCENTRATION (W.Q.O)

Table 1. Chlorophyll-a Concentration and its Percent Compositon by Size at Selected Stations of the Great Lakes, July 1980.

Station	Chlorophyll-a mg/m3	% Chlorophyll-a Nannoplankton <64-um	Netplankton >64-um	<10um	10-20um	20-64um
LAKE ERIE						
28 western basin	17.57	94.10	5.90	82.15	4.78	7.17
29 Bass Island	10.05	79.00	21.00	38.70	4.10	36.20
32 eastern basin	4.49	63.50	36.50	38.30	7.60	17.60
LAKE HURON						
25 Mississagi St.	1.10	84.70	15.30	77.48	1.80	5.42
26 Sulphur Island	1.38	85.51	14.49	70.29	0.72	14.49
27 Saginaw Bay	2.00	100.00	0.00	62.50	14.22	23.28
NORTH CHANNEL						
8 Serpent Harbour	1.16	83.62	16.38	77.54	0.00	6.08
GEORGIAN BAY						
5 Owen Sound	1.30	81.59	18.41	66.20	4.62	10.77
7 French River	1.01	91.09	8.91	69.01	22.08	0.00
LAKE SUPERIOR						
11 Grand Marais	1.16	75.00	25.00	71.64	3.36	0.00
13 Keewenaw Peninsula	0.97	86.60	13.40	61.18	25.42	0.00
16 Madeline Island	0.79	70.88	29.22	66.38	4.50	0.00
23 Superior Shoal	1.16	95.69	4.31	91.53	0.00	4.16

Table 2. Species Composition of Various Size Fractions of Phytoplankton and Their Percent Contribution at Station 13, Lake Superior, July, 1980.

Size Fraction	Species	Percent Biomass
<10 µm	Chroococus dispersus var. minor Smith	0.8
	Chlamydomonas globosa Snow	0.5
	Chlorella vulgaris Beyer	0.4
	Scenedesmus quadricauda var. quadrispina Smith	0.6
	S. bijugatus Kütz.	0.5
	S. hystrix Lagerheim	0.1
	Dictyosphaerium pulchellum Wood	1.4
	Ochromonas sphagnalis Conrad	1.3
	Ochromonas spp.	3.1
	Chromulina sp.	0.3
	Chrysochromulina parva Lackey	14.8
	Diceras chodatii Reverdin	0.2
	Stelexmonas dichotoma Lackey	0.1
	Uroglenopsis sp.	7.7
	Chrysamoeba sp.	4.6
	Salpingoeca frequentissima Teiling	0.2
	Kephyrion ovum Pascher	0.4
	Cyclotella sp.	0.9
	C. stelligera Grun.	1.6
	C. ocellata Pant.	4.3
	C. glomerata Bachmann	1.6
	C. atomus Hustedt	2.0
	C. botanica Eulensi	4.0
	C. comta Kütz.	3.6
	Rhodomonas minuta Skuja	2.4
	Katablepharis ovalis Skuja	1.0
10-20 µm	Oocystis submarina Lagerheim	0.4
	Elakatothrix gelatinosa Wille	0.1
	Dinobryon bavaricum Imhoff	0.8
	D. cylindricum Imhoff	0.3
	D. divergens Imhoff	0.4
	D. sertularia Ehr.	0.4
	D. sociale Ehr.	0.5
	Rhizochrysis sp.	2.0
	Fragilaria sp.	0.3
	Chroomonas breviciliate Nygaard	0.5
	Rhodomonas minuta Skuja	0.4
	Gymnodinium varians Haskell	1.2
20-64 µm	Ankistrodesmus falcatus var. spirilliformis West	0.05
	Mallomonas sp.	1.7
	Tabellaria flocculosa Kütz.	1.0
	Navicula cryptocephala Kütz.	0.8
	Achnanthes minutissima Kütz.	0.1
	Cryptomonas erosa Ehr.	1.8
	C. marssonii Skuja	2.9
	Peridinium pygmaeum Linden	3.5
	P. aciculiferum Lemm.	8.4
	Gymnodinium sp.	8.2
>64 µm	Ankistrodesmus falcatus Ralfs	0.1
	Asterionella formosa Hassall	1.6
	Nitzschia acicularis Kütz.	0.1
	Fragilaria capucina Desm.	0.8
	Synedra acus var. radians Kütz.	1.0
	Diatoma elongatum Agardh	0.5